promoter confers constitutive expression upon heterologous genes in most plants. Dissection of the intact promoter into subdomains that are able to confer tissue-specific gene expression demonstrated that the promoter has a modular organization. When selected subdomains are combined, they confer expression not detected from the isolated subdomains, suggesting that synergistic interactions occur among cis elements.

In other words, certain subdomains of the promoter were combined to confer tissue-specific expression. Additionally, this article makes reference to earlier work showing that a region of the 35S promoter from –90 to +8, relative to the transcription start site, called "domain A", has promoter activity (page 960): "Histochemical localization of expression shows that domain A confers expression principally in root or embryonic tissue destined to become root (12)." Reference is also made to earlier work with tobacco callus and leaf tissue, (page 960): "In tobacco callus and leaf tissue, deletion from –943 to –343 bp upstream of the transcription initiation site does not decrease expression. However, deletion to –105 decreases transcription to one-third of control values, and further deletion to -46 decreases transcriptional activity to one-twentieth of the amount observed with the –105 deletion (5)." Consequently, the Benfey et al. article teaches that very small regions of a promoter can still have functional activity in certain cell types. This article does not teach or suggest that sequence homology cannot be used to identify other promoters.

In contrast, the instant invention is concerned with an isolated nucleic acid fragment comprising:

a) a promoter having at least 80% identity with any of the nucleotide sequences set forth in SEQ ID NOs:6, 14, 15, or 16 based on the Clustal method of alignment, or

b) a promoter which hybridizes with any of the nucleotide sequences set forth in SEQ ID NOs:6, 14, 15, or 16 under moderately stringent conditions.

The claimed invention is not directed toward recombining subdomains in order to confer tissue-specific gene expression. Instead, the invention concerns constitutive SAMS promoters. Prior to the present invention, it was not known that constitutive SAMS promoters existed. In fact, such promoters do exist and can be readily isolated and used by one of ordinary skill in the art as is set forth in the specification and will be discussed hereinbelow.

The Kim et al. article concerned a 20-nucleotide **upstream** element, the "*nos* element", that was found to be essential for the nopaline synthase (nos) promoter activity. No promoter activity was found for an internal deletion mutant of the nos promoter, pGA939-126/-112, in which nucleotides –126 through –112, relative to the start of transcription, have been deleted. The *nos* **upstream** control region essential for promoter activity was studied by means of synthetic oligomers using transient and stable transformation systems. This article does not teach or suggest that sequence homology cannot be used to identify other promoters. This article teaches that promoter activity of a nucleotide sequence with known promoter function can be abolished by any of the following methods: (1) deletion of nucleotide sequences from

the 5' end until an essential promoter element is removed; (2) deletion of all or part of an essential promoter element from a larger DNA sequence; and (3) site-specific mutagenesis of a small number of nucleotides from within an essential promoter element. It is recognized that deletion and site-specific mutagenesis can abolish promoter activity. However, this would not be the case for isolation of a naturally occurring DNA sequence from genomic DNA, using the methods described in the specification and known to one skilled in the art. In the absence of manipulation of a naturally occurring DNA sequence, one would have a reasonable expectation that a DNA fragment with sequence homology to a SAMS promoter would also have promoter activity. In this regard, we refer Example 3 of the specification, which employed SEQ ID NO:6 for a DNA hybridizaiton experiment. The result of this experiment is summarized on page 12, lines 13 - 15: "Southern hybridization analysis of soybean genomic DNA using a 1314 base pair DNA fragment from upstream of the SAMS protein coding sequence as a probe indicated that this fragment is unique in the soybean genome (Figure 1B)." By way of example, but not limitation, if genomic DNA from other cultivated varieties of soybean, or from wild relatives of soybean, were hybridized to SEQ ID NOs:6, 14, 15 or 16, a DNA fragment with positive hybridization would be expected to have promoter activity.

It is respectfully submitted that neither of these articles, either alone or in combination, supports the proposition that sequence homology cannot be used to identify other promoters as recited in claim 1 of the present invention. In fact the Kim article noted that **substitution** of the *nos* element with the *ocs* or 35S *as-1* which contain **similar** hexamer motifs **restored** not only promoter activity but also responses to wounding auxin, methyl jasmonate and salicylcic acid. In other words, similar (homologous) motifs could be substituted for the *nos* element and restore activity as well as responses to wounding auxin, etc. This suggests that sequence similarity can be useful in identifying other sequences having similar function.

Submitted herewith is the Declaration of Dr. Zhongsen Li which shows that another DNA fragment (SEQ ID NO:14) functions as a promoter in a transgenic cell or plant.

One method to obtain sequences similar to the SAMS promoter is described in Example 4, page 19, lines 9 – 31, in which a fragment corresponding to SEQ ID NOs:6, 14, 15 or 16 would be substituted as a hybridization probe, in place of the 315-bp SAMS cDNA fragment:

> "This PstI fragment was labeled with -^{32}P-dCTP, as described in Example 3, and used as a probe to screen a soybean genomic DNA library that had been constructed in a EMBL3 SP6/T7 vector (ClonTech, Palo Alto, CA). The library was plated with LE392 (ClonTech) cells at 50,000 plaque forming units (pfu) per 150 mm NZCYM agar plate (GIBCO BRL). Plaques were transferred to Hybond nylon membranes, and the plaque replicas were then denatured and neutralized according to the manufacturer (Amersham Life Science, Arlington

> Heights, IL). The phage DNA was fixed on the membranes by UV-crosslinking (Stratagene). After prehybridization at 65 for 1 hour in 0.5 M $NaHPO_4$, pH 7.2, 1 mM EDTA, 1% crystalline BSA (Sigma), and 7% SDS, the SAMS 315 bp Pst1 fragment probe was denatured in boiling water bath for 5 minutes and added to the same hybridization solution, and was hybridized at 65 for 24 hours. The membranes were washed in 40 mM $NaHPO_4$, pH 7.2, 1 mM EDTA, 0.5% crystalline BSA, and 5% SDS for 10 minutes at room temperature, and then 3x 10 minutes at 65 in 40 mM $NaHPO_4$, pH 7.2, 1 mM EDTA, and 1% SDS. The membranes were exposed to Kodak X-ray film (Sigma) at -80 . Positive SAMS genomic DNA phage clones were suspended in SM buffer, 50 mM Tris-Cl, pH 7.5, 100 mM NaCl, 0.2% $MgSO_4 \cdot 7H_2O$, and 0.1% gelatin, and purified by a secondary screening following the same procedure. Twenty three strongly hybridizing plaques were identified by the first screening from a total of $3x10^5$ pfu, and fifteen were later purified. DNAs were prepared from two of the purified phage clones (Ausubel et al., (1990) pp. 1.13.4-1.13.8), they were digested with BamHI, ClaI, PstI, and NcoI and prepared for a Southern blot. The blot was hybridized with the SAMS 315 bp PstI fragment probe prepared and used as above. A single positive fragment of clone 1 was identified from the ClaI digestion."

In additional to substitution of SEQ ID NOs: 6, 14, 15 or 16 for the 315-bp PstI fragment as a probe, the genomic DNA library to be screened could correspond to genomic DNA from a cultivated variety of soybean, a wild relative of soybean, or a plant unrelated to soybean.

After a nucleotide sequence has been identified which meets the sequence identity criteria, then promoter activity can be assessed in the following manner:

(1) Construction of a gene expression cassette containing the relevant polynucleotide sequence 5' linked to a reporter gene is shown in Example 5, page 21, in which an expression cassette is constructed containing the SAMS promoter 5' to the GUS reporter gene:

> The activity of the soybean SAMS promoter was tested by its ability to express the GUS reporter gene in transgenic *Arabidopsis* plants carrying the SAMS promoter::GUS::3' Nos expression casstette. GUS refers to the *E. coli* -g lucuronidase gene (GUS) (Jefferson, (1987) *Plant Mol. Biol. Rep.* *5*:387-405) and 3' Nos refers to the transcription termination region from the nopaline synthase (Nos) gene (Depicker *et al.* (1982) *J. Mol. Appl. Genet.* *1*:561-570). The SAMS promoter fragment (SEQ ID NO:6) was digested with XbaI and NcoI and inserted into plasmid pMH40Δ (SEQ ID NO:17), which contained a 35S promoter::GUS::3' Nos plant expression cassette. The XbaI/NcoI SAMS promoter DNA fragment replaced the 35S promoter of pMH40Δ, to form the pZSL11 plasmid (Figure 3). The SAMS promoter::GUS::3' Nos DNA fragment (SEQ ID

> NO:18) was excised from pZSL11 by HindIII and SacI digestion and transferred into the corresponding sites of pBI101 (ClonTech) binary vector.

Once the gene expression cassette has been made, then next step involves (2) transformation of a cell with the gene expression cassette.

An example of stable transformation of *Arabidopsis* using *Agrobacteria* mediated transformation is given in Example 5, page 21:

> The SAMS::GUS expression cassette was introduced into wild type *Arabidopsis thaliana* by *Agrobacteria* mediated transformation. *A. thaliana* ecotype columbia were grown in 228 chamber with continuous light and transformed by vacuum infiltration method using GV3101 *Agrobacteria* (Bent, A. *et al.*, (1994) *Science 265*:1856-1860). Transformed *Arabidopsis* seeds were selected by germination on Murashige and Skoog minimal salt (GIBCO BRL) plus 0.2 % phytagel (Sigma), 1% sucrose, and 100 mg/ml kanamycin. The kanamycin resistant seedlings were transferred into soil and grown in 228 chamber under continuous light.

Alternatively, one can utilize transient transformation of plant tissue by particle bombardment (Klein et al. (1978) *Nature* (*London*) 327:70-73; U.S. Patent No. 4,945,050).

These techniques are well known to those of ordinary skill in the art. No undue experimentation is required in order to practice transformation.

Having transformed plant cells in either a stable or transient fashion, one can then proceed with step (3), assay of reporter gene linked to the fragment of interest. When the reporter gene is GUS, it can be detected using a variety of methods well known to those of ordinary skill in the art, such as, histochemical staining and fluorescence analysis. For the histochemical GUS staining assay, GUS activity is correlated with the appearance of blue-staining cells. The details of the histochemical GUS staining assay are given in Example 5, page 22:

> For histochemical GUS staining, plant tissues were incubated in 0.5% 5-bromo-4-chloro-3-indoxyl- -D-glucuronic acid (X gluc, Biosynth AG, Switzerland) in 50 mM sodium phosphate, pH 7.0, 10 mM EDTA, 0.5 mM potassium ferricyanide, and 0.5 mM potassium ferrocyanide at 378 overnight, and then chlorophyll was removed with 75% ethanol. Pictures were taken using a Nikon dissecting microscope.

An analytical measurement of GUS enzyme activity can be obtained by a fluorometric assay. The details of the GUS fluorescence assay are given in Example 5, page 22:

> For fluorescence analysis, plant tissues were ground in microfuge tubes with extraction buffer, 50 mM phosphate buffer, pH 7.0, 10 mM EDTA, 0.1% Triton X-100, 0.1% N-lauroyl sarcosine, and 10 mM -m ercaptoethanol, to homogeneity. The samples were centrifuged at 14,000 rpm for 10 minutes, and aliquots of the supernatant were used to determine protein concentrations by the Bradford method (Bio-Rad, Hercules, CA) using 96 well microtiter plates read with a kinetic microplate reader (Molecular Devices, Sunnyvale, CA). The -g lucuronidase activities were analyzed by standard protocol (Jefferson *et al,* (1987) *EMBO J.* *6*:3901-3907) using 96 well microtiter plates read with Cytofluor multiwell plate reader (PerSeptive Biosystems, Framingham, MA).

Thus, by following the steps described in the specification, as discussed above, one of routine skill in the art can readily determine if a given polynucleotide sequence has promoter activity in plant cells.

In view of the above discussion, it is respectfully submitted that there is no lack of written description and that to the extent that some experimentation may be required, such experimentation would be routine and well within the level of skill in the art.

Accordingly, withdrawal of the rejection of claims 1-7 under 35 USC §112 is respectfully requested.

Claims 1-11 stand rejected under 35 USC §112, first paragraph, "because the specification while being enabling for an isolated nucleic acid fragment comprising a promoter that is SEQ ID NO:6, does not reasonably provide enablement for an isolated nucleic acid fragment comprising a promoter having at least 80% identity with any of the nucleotide sequences set forth in SEQ ID NOS:6, 14, 15, or 16. . . .Given the unpredictability of a particular isolated nucleic acid fragment functioning as a promoter, the absence of guidance in the specification for making and using the claimed promoters, the lack of working examples, and given the breadth of the claims which encompass promoters having at least 80% identity with any of the nucleotide sequences set forth in the SEQ ID NOS:6, 14, 15, or 16, and promoters which hybridize with any of the nucleotide sequences set forth in SEQ ID NOS:6, 14, 15, or 16, as well as plants and seeds comprising said promoters, it would require undue experimentation by one skilled in the art to make and/or use the claimed invention."

It is respectfully submitted that this ground of rejection is not apposite for all of the reasons discussed above with respect to the previous rejection. Accordingly, withdrawal of

the rejection of claims 1-7 under 35 USC §112 is respectfully requested. Claim 7 has been amended to address the rejection under 35 USC §101.

In view of the foregoing discussion and amendments, Applicants respectfully request withdrawal of the rejection of the claims under 35 USC §112, first paragraph. It is respectfully submitted that the claims are now in form for allowance which allowance is respectfully solicited.

The Declaration of Dr. Li and the Version With Markings to Show Changes Made accompany this response. Changes in the version with markings are indicated as follows: deletions are bracketed and insertions are underlined.

A petition for a one (1) month extension of time also accompanies this response.

Should any fee be required in connection with the filing of this response, then please charge such fee to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Respectfully submitted,

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Version With Markins to Show Changes Made

Changes are shown as follows: deleted material is bracketed and inserted material is underlined.

7. (twice amended) Seed of the plant as in any one of Claims 3-6 <u>wherein said seed comprises in its genome the chimeric gene of claim 2</u>.